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CHEMISTRY OF DIATOMIC ALGAE (DIATOMEAE)

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Chemistry of Diatomic Algae (DIATOMEAE)

by

G. K. Barashkov

Diatomic algae are widely distributed in nature, especially in seas, where they form enormous masses of organic substance - about a half of the amount synthesized on the globe within a year.

They have a brownish color with various shades of yellowness and greenish tinges. This is due to the presence in them of a complex of pigments additional to chlorophyll, known under the general name of diatomin (diatomite). The characteristi: difference of diatomeae from other algaes is the shell of various degrees of silification, consisting of two halves. The component part of the shell are considere pectin substances, and the product of assimilation and a reserve substance - oil (Zhuze, Proshkina-Lavrenko, Sheshukova, 1949).

Development of diatomic algae in seas of moderate and high latitudes assures approximately tenfold surpassing of primary production and biomasses of the plankton in these latitudes as compared with tropical zones (Bogorov, 1958). A generally adopte opinion about the diatomic phytoplankton as a source of feeding plankton crustacea, basically copepoda, which in turn are consumed by much larger animals (Zernov, 1934; Zenkevich, 1951).

The interest to the chemistry of diatomeae is determined not only by such first rate important problems, as photosynthesis and biological productivity of water reservoirs, but also by the value of deposition accumulation in water reservoirs and conversions of these sedimentary organic substances.

In recent years there is a noticeable increase in the interest of various scientists, including biochemists, in diatomeae, a number of reports has appeared, consi-

derably enlarged our ideas about these algae. So far there are no reviews of books dealing in the chemistry of diatomic algae. This report appears to be an effort to fill in this gap.

Carbohydrates

Mono- and oligosaccharides. The chemistry of carbohydrates of diatomeae was being investigated only since 1952 at the Moscow University. G.K.Barashkov showed that glucose and several oligosaccharides transform into an alcohol extract of diatomic phytoplankton. One of these saccharides consists of two, the other one of three glucose radicals (Barashkov,1956). The glucose content after three monthly storing of assembled samples in an 80%-alcohol reached 8% of the weight of the organic part of the material; the content of oligosaccharides in such algae varied within 14-16% of the organic part (Serenkov and Barashkov,1954).

The detection on chromatograms of several oligosaccharides, consisting of glucose, made it possible to assume, that oligosaccharides of diatomeae appear to be the product of decomposition of a more complex compound. It can, apparently, transform into high concentration alcohol with gradual hydrolysis into glucose.

Polposes. Until recently literature had no information on the chemical analysis of any one carbohydrate or polysaccharide of diatomic algae. Polysaccharides of diatomeae were conjectured only by results of qualitative reactions.

Frenzel (1897) showed that under the silicon shell Melosira nummuloides there is an organic shell, presumably consisting of pectin substances. Then Mangin (1908) on the basis of positive coloring the shell of algae protoplasm Biddulphia sinensis with an ammonia solution of Ruthenium trichloride (better known under the name red Ruthenium) also made an assumption about the presence of pectin substances in diatomeae. The shell of cells of these algae was also colored with methylene blue, saframine, neutral red. Liebisch (1929) repeated Frenzel's and Mangin's experiments, coloring the shell of representatives of various types of algae with methylene blue. He also

arrived at a conclusion that the shell of these diatomes consists of pectin substances.

In monographies and textbooks, devoted to diatomic algae, this point of view about the presence in them of a pectin shell was being adopted as a proven fact (Arnoldi, 1925).

But gradually data began accumulating on the fact that positive qualitative reactions with red ruthenium, alike pectin substances, may also yield polysaccharides, containing free carboxyls, such as gum, hemicellulose (Kohler, 1906; Bonner and Preston 1949). It was explained, that the solution of red ruthenium may color coagulated protoplasma and cellular nucleus, knowingly containing no pectin substances. A positive reaction with this reagent is offered by certain lipides. It was found, that also other basic dyes, such as methylene blue and safranine, can also color the most variegated substances (Kerr and Bailey, 1934). In this way, the presence of pectin substances in diatomes has been proven with the aid of far not without fault qualitative reactions. And relatively recently (Chadefaud 1949) by positively coloring the cells of sea diatomes Licmophora lyngbiei with vanillin chlorohydrate concluded, that it contains "fucosan" substance, which is known in the role of polysaccharide of brown algae.

As result of chemically analyzing a mixture of sea diatomic algae of the Far East, algae consisting of 85-90% of radial plankton algae Thalassiosira nordenskioldii and Th. gravida, it was explained, that they do not contain any cellulose, hemicellulose, similar to the ones in higher plants, and starch. Also no saccharose and other fructosides have been found. The carbohydrates in the amount of 3-5% of the weight of the organic part of the algae were found to be polysaccharides of the dextrin type (Barashkov and Barashkov, 1954). At a total content of carbohydrates in the investigated samples in the order of 26-30% of the organic algae substances, i.e. 12-15% of dry weight, such a low content of polysaccharides led to the assumption, that they can hydrolyze in alcohol of high concentration, reaching 80%. This may well explain the high content of glucose in free form and alcohol-soluble carbohydrates. This is also confirmed by experiments with redeposition of polysaccharide, separated by extraction from

algae using cold water. Losses of carbohydrates in samples after redeposition reached 80-90% of the initial amount (Barashkov, 1956).

In the mentioned experiment in hydrolyzate samples of polysaccharide was revealed glucose, galactose, xylose, rhamnose, arabinose or mannose and an unindentified sugar. Quantitative paper chromatography showed, that polysaccharide consists of 60-80% of glucose. A considerable part of it is constituted alse by xylose, remaining sugar was in insignificant amount. Uronic acids in the compounds have not been detected neither by the chromatographic method, nor when employing the qualitative reaction with carbazole. These data indicate the absence in the investigated algae of pectin substances, which appear to be, as is known, polyuronides.

In addition to the experiments carried out by us in 1958, were published also results of analogous investigations in representatives of two-sided diatomeae - sea Amphipleura rutilans (Lewin, 1958) and obtained from artificial culture Phaeodactylum tricornutum (Lewin, Lewin and Philpott, 1958) which the authors consider as belonging to diatomeae. The results of these investigations also confirm the absence in the investigated algae of pectin substances. In the A. rutilans hydrolysate were detected xylose, mannose, traces of rhamnose and two unindentified sugars with low mobility on the chromatogram. Neither uronic acids, nor hexoseamine have been detected. A similar analysis of hydrolysates of mucilaginous capsular material of oval cells of P. tricornutum, soluble in hot water, showed the presence in it of xylose, mannose, fucose and galactose. In addition to proving the absence of pectin substances in all enumerated algae, the mentioned analytical investigations give proof, that each type of algae has a collection of sugars, differing from same in other algae.

There are data, confirming the formation in diatomeae, under certain conditions, of specific pectin substances. And so in a two-sided Navicula pelliculosa algae, germinated artificially at insufficient content in the culture medium of certain, necessary for normal vitality, elements, such as silicon, nitrogen of phosphorus, was detected the formation of a jelly-like capsule around the cells. This capsule was well

soluble only in a 20%- solution of sodium hydroxide. Analysis has shown the presence in it basically of glucouronic acid (Lewin,1955a).

When investigating the autolysis processes in various planktonic algae with the aid of luminescent microscopy S.V.Goryunova (1958) found, that the silicon armors of Coscinodiscus type cells, in contrast to remaining plankton diatomeae, during the bombardment with ultraviolet light possess a bright bluish-greenish luminescence. This is due to the fact, in the opinion of the author, that algae of this type contain specific organic substances of the pectic acid type.

These investigations indicate that in some instances diatomeae, apparently, can form mucilaginous substances of carbohydrate nature of the type of specific pectinic substances, which possess exceptionally high stability to chemical reactions.

A greater content of substances of carbohydrate nature, reaching 30 and more percentages of organic algae substance, forces us to make still another assumption, differing from the generally adopted ideas. As has been mentioned, the product of assimilation and the reserve substance of diatomeae is considered to be oil. On the basis of above mentioned data about the quantitative content of carbohydrates in algae (diatomeae), it is possible to assume, that carbohydrates can be reserve substances of these algae. This assumption gains substantial confirmation in the fact, that cells of Phaeodactylum tricornutum algae, situated in various stages of development (oval and spindle-shaped), differ sharply from each other in the content of carbohydrates. oval cells contain 22%, and spindle-shaped - 2% of carbohydrates of the total amount of organic substances (Lewin, Lewin and Philpott,1958). Such a change in the amounts of any given substance during the development cycle is ordinarily considered as a sign, characteristic for reserve substances.

Nitrogen-containing Substances

About the content of nitrous substances in diatomic algae and idea can be gained from the quite sparse data on the determination of total nitrogen,shown in table 1.

Table 1.

Content of nitrogen in diatomeae (in percentages of dry weight)

Type	Nitrogen	Literature source
Navicula pelliculosa	5.05	Collyer a. Fogg, 1955
Mixture - Chaetoceros, Rhizosolenia, Coscinodiscus	2.10	Vinogradov, 1938
Mixture - Rhizosolenia, Sceletonema, Thalassiotrix	4.45	
Mixture - Thalassiotrix, Sceletonem, Chaetoceros	3.33	
Mixture - Thalassiosira (90-95% of total mass	4.34	Seremkov and Barashkov, 1954
Mixture - Thalassiosira (75-80%) Chaetoceros Furcellatus, Fragilaria, Oceanica	5.08	

It is evident from this table, that mainly mixtures of sea diatomeae have been subjected to investigation. The average amount of nitrogen constitutes more than 3%. Since the investigated sea algae have a greater ash content (more than 50%), it should be concluded, that the organic part of the algae is rich in nitrous substances.

Analysis of a mixture of Far Eastern planktonic sea diatomeae, consisting of 90-95% of representatives of Thalassiosira type, showed the presence in them of 29.09% albumina when figuring the organic part of the cells. A similar analysis of a more variegated mixture of diatomeae showed the presence in them of 36.1% albumina in ratio to the organic part (Serenkov and Barashkov, 1954). There are also data about a greater content of albumina in diatomeae. For example, the calculated amount of albumina in oval cells of Phaedodactylum tricornutum equalled 39%, and in spindle shaped 47% in ratio to the organic part of the cells (Lewin, Lewin and Philpott, 1958).

In the investigated mixtures of Far Eastern phytoplankton almost one half of the total nitrogen was nonalbuminous nitrogen (2.12%) nonalbuminous and 4.34% total in the first sample and 2.39 and 5.08 respectively in the second one). In this way, in this respect diatomic algae, as well as a number of other types of algae, differ from green ones, in which nonalbuminous nitrogen constitute on an average 1/10 part of the total. A considerable part of the nonalbuminous nitrogen was found to be

soluble in ether, i.e.it belongs to lipides.

The study of the nature of nonalbuminous nitrous substances showed that a certain of their part is made up by amino acids:aspartic, glutamic acids, glycine, alanine, tyrosine. The amount of free amino acids reached 0.2 - 0.3% of dry weight (Serenkov and Barashkov,1954).

In the composition of albumins have been detected amino acids ordinary for plants, including all irreplaceable. The greatest part of the albumin was made up of aspartic and glutamic acids (33-39%) as well as of lysine and proline.

Quantitative analyses of nucleinic acids showed, that RNK and DNK in toto constitute about 5% of dry weight. Purine nucleotides were found in somewhat greater amount than pyrimidine nucleotides (1.0 : 0.7) Serenkov and Pakhomova,1955).

The data by Low 1955, confirm the presence in diatomeae of ordinary amino acids. Differences in qualitative composition of amino acids in sea and fresh water algae have not been detected, but oxyproline was found only in fresh water Nitzschia palea. After separating nucleinic acids and analyzing same was detected a certain prevalence of purines over pyrimidines. In fresh water algae Navicula pelliculosa and Nitzscia palea the ratio of purines to pyrimidines was 10.3 and 1.04 respectively, and in sea Cylindrotheca gracilis 1.01. In all instances the qualitative composition of RNK and DNK did not deviate from typical compounds of these acids made from other sources. 5-Methylcytosine has not been detected (Low,1958).

Lipides

Lipides appear to be the most investigated diatomeae substances, they are easily extracted from cells and are ordinarily contained in greater quantities. The quantitatively soluble in benzene fraction Navicula pelliculosa was 15-35% of dry weight (Collyer and Fogg,1955; Fogg,1956). In Nitzschia sp algae in artificial culture,the content of lipides was lower, reaching only 9.5% (Deuticke,Kathen and Harder,1949). On the average, taking into consideration a greater amount of ashes in diatomeae, it

is possible to assume, that they contain about 20% of lipides. For example, a mixture of sea diatomeae had 10% of lipides at 50% ash, another mixture contained 15% lipides at 36% ash. A mixture consisting of 75% Rhizosolenia closterium had 8% lipides at 46% ashes, and artificial culture Nitzschia closterium had about 5% lipides at 42% ashes (Clarke and Mazur,1941). A mixture of sea planktonic Far Eastern diatomeae,constituting 90-95% of Thalassiosira type algae had 15% lipides at an ash content of 52.3%, and in mixture of Thalassiosira (75-80%), Fragilaria oceanica (5-7% and Chaetoceros furcellutus (10-15% was found 16% of lipides at an ash content of 53.2% (Serenkov and Barashkov,1954).

The total amount of lipides, produced by diatomeae, is considerably greater, since about 10% of the dry weight of algae cells are constituted by lipides,formed alive in the medium. In a culture medium, in which Synedra sp has been germinated S.V.Goryunova (1954) were detected considerable quantities of lignoceronic acids.

Fats constitute in Nitzschia lipides 80.5%. Among triglycerides were detected the following: 1) glycerin-stearic acid, glycerin-oleic, glycerin-linoleic, 2) glycerin-linoleic, glycerin-stearic. The iodine number of this fat reached up to 116 (Deuticke and others 1949; Kathen,1950).

Judging by the acid composition Nitzschia closterium fats represented a mixture consisting of saturated acids with C_{14} 8%, C_{16} 17%, C_{18} 2% and from unsaturated acids from C_{14} 1%, C_{16} 36%, C_{18} 20% and C_{20} 16%. In this way,the basic mass of acids is represented by unsaturates (Lovern,1936). Approximately the very same ratio of saturated and unsaturated acids is indicated by Clarke and Mazur,but they detected more high molecular acids. And so, of all unsaturated acids per fraction of the acid from C_{16} 26% was from C_{22-24} 31% and from C_{26-30} 11%.

Determination of the amount of triglycerides only by the saponification results for diatomeae lipides does not appear to be without errors. This was shown by Clarke and Mazur 1941, on an artificial culture of Nitzschia closterium and on a mixture of

sea diatomeae as well. It was explained, that in freshly prepared algae there were somewhat more of free fatty acids, than bound ones. The composition of diatomeae lipides, found by them, is given in table 2.

It is evident from the data of this table that the amount of free fatty acids decreases during storage. A study of this phenomenon showed that after a six month storage the content of free fatty acids in the lipides decreases, but the amount of hydrocarbons increases. Clarke and Mazur consider this phenomenon as highly important from the viewpoint of petroleum formation. In a nonsaponified fraction they found a product of the sitosterol type.

Table 2. Composition of diatomeae lipides according to Clarke and Mazur (in %)

Fraction of lipides	Nitzschia closterium	mixture of diatomeae 25 %	mixture of sea diatomeae	mixture of diatomeae within 5 days after [illegible]
Free fatty acids	80	82	70	59
Bound fatty acids	17	5	1	17
Non-saponifying fraction	—	12	29	—
Alcohols	3	—	—	7
Hydrocarbons	3	—	—	14

About the quite larger content of sterols in Navicula pelliculosa, of the order of 0.01 - 0.06% of dry weight, was reported by Low, 1955. It identified the basic component as chondrillasterin.

Pigments of diatomeae are listed in the resume by Cook, 1945. He noticed the presence of chlorophyll A and C (chlorofucine or chlorophyll γ), β-carotine, fucoxanthine, and pigments, constituting the diatomeae (neofucoxanthine, diatoxanthine and diadicoxathine). Apparently, diatomeae have chlorophyll compounds -albumina-fucoxanthine. To the very same conclusion came Wassink and Kersten, 1947) when studying light absorption by alcohol extracts and live cells of Nitzschia dissipata.

Analogous compunds allow with the aid of an energy migration mechanism to transmit the energy of quantum absorption to the necessary place not only from chlorophyll molecules, but also from molecules of other pigments. This is of greater biological importance, because it increases by many times the

effectiveness of photosynthesis on account of adopting the energy of the greater additional zone of the spectrum.

Other Investigations

Ash content. As already mention the content of ashes in diatomic algae is very high and reaches up to one half of the dry weight of cells, and it is even higher. In fresh water forms the content of ashes is, as a rule, smaller, than in sea algae.

It is known, that the diatomeae shell contains silica, close in composition to opal (Arnoldi, 1925). It is therefore perfectly natural, that to the exchange of Si in diatomic algae was devoted an extensive number of investigations.

Tho content of silicic acid in shells of 15 investigated forms of diatomeae was highly variegated and inversely proportional to the number of cells in the population (Einsele and Grim,1938). These data, obtained on planktonic material, were then confirmed on Nitzschia palea cultures, cultures of Bacillaria paradoxa and Thalassiosira nana (Jorgensen,1955). Planktonic forms in lakes of Danemark show such a dependence (Jorgensen 1953). Jorgensen assumes that diatomeae as they grow form in the medium certain substances, inhibiting the assimilation of SiO_2, i.e. there is self-poisoning of this process in limited volume of the medium. These observations should be accepted during culturing of diatomeae for the purpose of obtaining a greater number of material.

It is apparent, that silicon plays a much greater role in the vitality of algae, and this not only of diatomic, which is ordinarily considered as a fact accompli. In addition to the mentioned investigations by Jorgensen, to such a conclusion can one arrive on the basis of recent investigations by Ryther and Huillard,1959. They have shown, that the addition of silicon salts to sea water, containing natural phytoplankton, results in a considerably greater increase in rate of photosynthesis in regions poorer in nutritious salts in the ocean, than the addition of phosphorous or nitrous salts.

The structure of silicic acid in diatomeae shells has, apparently, an amorpho-

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us nature. To such a conclusion came Helmke when investigating shells of diatomeae with the aid of an electron microscope. Consequently, it can be assumed that such a shel has greater absorbability and it probably participates more actively in the metabolism between protoplasma and surrounding medium (Helmcke,1954).

Assimilation of silicic acid by cells of Navicula pelliculosa diatomeae is connected with the process of aerobic breathing. At partial appearance of silicon absorption. the ability toward its assimilation is restored by adding to the medium sulfur containing compounds, such as glutathione, I-cysteine,methionine, sodium thiosulfate and sodium sulfide, as well as a mixture of sulfate and ascorbic acid (Lewin,1954). Experiments on the effect of poisons on respiration and silicon absorption confirmed the relationship between the two processes (Lewin,1956).

In addition to silicon the mixture of diatomeae Seeletonema, Thalassiotrix and Chaetoceros showed the presence of iodine in the amount of 0.003 - 0.023% of dry weight of cells. A.P.Vinigradov (1938)assumes, that iodine in the fat of fish has a "diatomic" origin and is accumulated as result of the nutritional chain phytoplankton- zoooplankton-fish existing in the sea.

Of other substances it is possible to notice the presence in diatomeae of Fragilaria and Navicula types of volutin-polyphosphates. No nucleoproteides have been discovered ib volutin (Keck and Stich,1957).

Very poorly investigated were conversions which take place with diatomeae after their dying off, although they are of considerable interest. As result of investigating the process of mineralization of residues of planktonic algae it was explained that in the first 11 months is liberated almost the entire nitrogen, but calcium remains almost in toto bound in the cells (Kleerekoper, 1953).

Summary

A review of available literature shows that there are not too many reports dealing in chemistry and biochemistry of diatomic algae.

The albumina content, according to available data,constitutes in algae cells

is 20-30%, 5-20% lipides, 12-20% carbohydrates and 20-60% ashes of dry weight. The greater content of ashes, quite sharply changing in various algae, compels one in acknowledging as adviseable to express the results of analyzing diatomeae in percentages of the amount of the organic substance. In this case it is possible to judge more objectively about the chemical composition of algae.

Sea diatomeae in the period of mass development contain easily hydrolizable polyglucoside, and not pectin substances, as it has been considered until now. At the same time in their composition are encountered many nitrogen containing substances, of which about one half of the total nitrogen cells goes for nonalbuminous substances.

In the composition of lipides, having an unsaturated nature, up to 80% of fatty acids can be found in free form. If it is assumed, that the content of lipides in ratio to the general weight of organic diatomeae substances constitutes in planktonic algae 30%, then we will deviate much from the true value. The amount of energy of 100 g of organic substances of the cells constitutes 525 cal. This value raise the caloricity of such a highly nutritive product, as chocolate .

The above mentioned review shows clearly, that the enormous importance of diatomic mic algae for subsequent organisms in nutritional chains of water reservoirs is not accidental. These algae in the period of mass development have in their composition substances, which together with high caloricity, hydrolyze easily and are well adopted, ted, representing a full values food. This pertains also to carbohydrates, and albumina, mina, and lipides.

No one should think however, that all diatomeae always have substances of identical chemical composition, which possess high nutritive value. For example, I.S. Gayevskaya, 1947, considers, that Caspian diatomeae Rhizosolenia calcar-avis does not have a positive effect on the biological productivity of the sea, although it gives greater values of primary production, because its cells are not in great demand by planktonic crustacea. As mentioned in review of reports dealing in carbohydrates some algae can form under unfavorable conditions specific polysaccharides, which

appear to be extremely stable to chemical actions. Such substances hardly have any greater nutritional value. But they no doubt play a greater role in nature, particularly in processes of deposition accumulation, where they may appear in the role of binding substances.

The peculiarity of the chemical composition and interchange of diatomic algae, the great importance in their vitality of silicon and sulfur, the presence of lipides in role of reserve products etc.- all this attracts special attention of biochemists to diatomeae. But right now the basic hindrance in studying this type of plants, which apparently, plays an important role in the natural processes of the globe, is the greater difficulty in the obtainment of sufficient quantities of material for analyses. Direct investigation on board larger expedition ships in places where the sea blooms appears to be an important step in furthering the study of diatomic algae. In this connection greater attention should be devoted to the adoption of methods of culturing these algae in laboratory conditions.

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Polar Scient.Res.Inst.of Sea Fish Economy and Oceanography named after N.M.Knipovich at Murmansk.

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